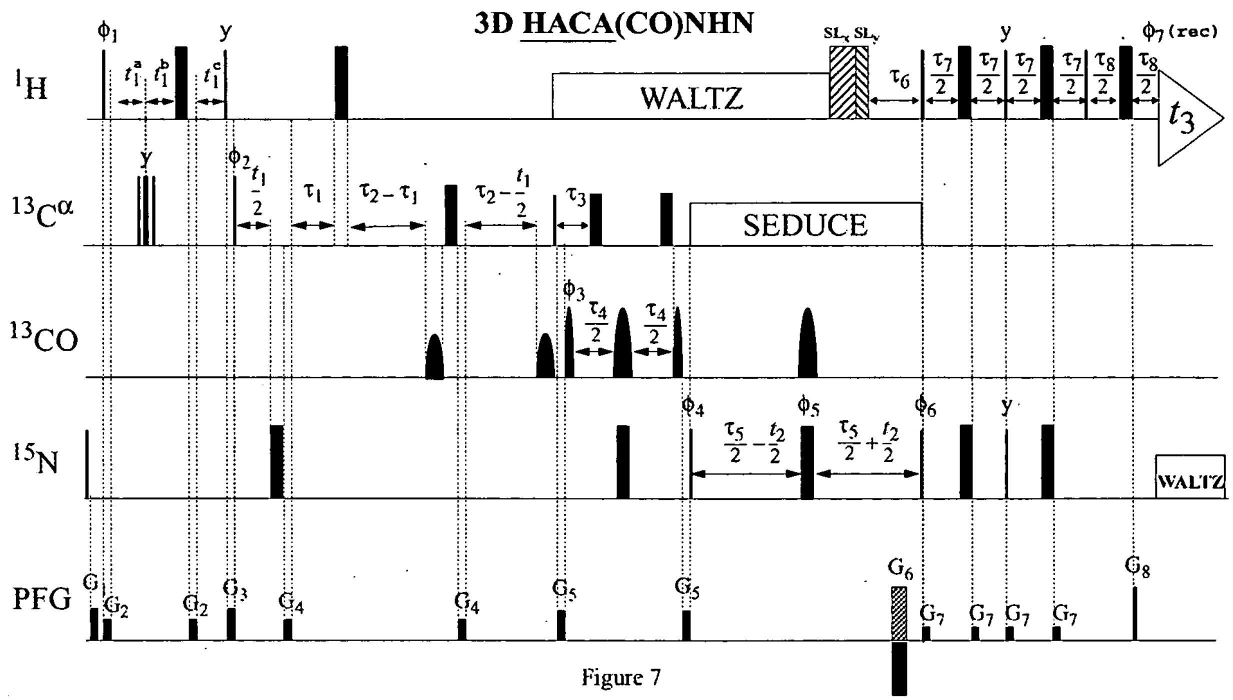
Figure 2B

Supporting Information for Szyperski *et al.* (2002) *Proc. Natl. Acad. Sci. USA* **99** (12), 8009–8014. (10.1073/pnas.122224599).

Supporting Figure 7

Fig. 7. Experimental scheme for the 3D HACA(CO)NHN experiment. Rectangular 90° and 180° pulses are indicated by thin and thick vertical bars, respectively, and phases are indicated above the pulses. Where no radio-frequency (rf) phase is marked, the pulse is applied along *x*. The scaling factor k for ^{1}H chemical shift evolution during t_1 is set to 1.0. The high power 90° pulse lengths were 5.8 ms for ^{1}H and 15.4 ms for ^{13}C, and 38 ms for ^{15}N. Pulses on ^{13}C prior to $t_1(^{13}C)$ are applied at high power, and ^{13}C decoupling during $t_1(^1H)$ is achieved using a $(90_x\text{-}180_y\text{-}90_x)$ composite pulse. Subsequently, the 90° and 180° pulse lengths of $^{13}C^a$ are adjusted to 51.5 and 46 ms, respectively, to minimize perturbation of the ^{13}CO spins. The width of the 90° pulses applied to ^{13}CO pulse is 52 ms and the corresponding 180° pulses are applied with same power. A SEDUCE-shaped 180° pulse with a length 252 ms is used to decouple ^{13}CO during t_1. The length of the spin-lock purge pulses SL_x and SL_y are 2.5 ms and 1 ms, respectively. The WALTZ16 composite pulse decoupling scheme is employed to decouple ^{1}H (rf field strength = 9.2 kHz) during the heteronuclear magnetization transfers as well as to decouple ^{15}N during acquisition (rf = 1.78 kHz). The SEDUCE sequence is used for decoupling of $^{13}C^a$ during the ^{15}N chemical shift evolution period (rf = 1.0 kHz). The ^{1}H rf carrier is placed at 0 ppm before the start of the semiconstant time ^{1}H evolution period, and then switched to the water line at 4.78 ppm after the second 90° ^{1}H pulse. The $^{13}C^a$ and ^{15}N rf carriers are set to 56.1 and 120.9 ppm, respectively. The duration and strengths of the pulsed z-field gradients (PFGs) are: G1 (1 ms, 24 G/cm); G2 (100 ms, 16 G/cm); G3 (1 ms, 24 G/cm); G4 (250 ms, 30 G/cm); G5 (1.5 ms, 20 G/cm); G6 (1.25 ms, 30 G/cm); G7 (500 ms, 8 G/cm); G8 (125 ms, 29.5 G/cm). All PFG pulses are of rectangular shape. A recovery delay of at least 100 ms duration is inserted between a PFG pulse and an rf pulse. The delays are: t_1 = 1.6 ms, t_2 = 3.6 ms, t_3 = 4.4 ms, $t_4 = t_5$ = 24.8 ms, t_6 = 5.5 ms, t_7 = 4.6 ms, t_8 = 1 ms. ^{1}H-frequency labeling is achieved in a semiconstant-time fashion with $t_1^a(0)$ = 1.7 ms, $t_1^b(0)$ = 1 ms, $t_1^c(0)$ = 1.701 ms, Dt_1^a = 60 ms, Dt_1^b = 35.4 ms, and Dt_1^c = -24.6 ms. Hence, the fractional increase of the semiconstant-time period with t_1 equals to $l = 1 + Dt_1^c/Dt_1^a = 0.58$. Phase cycling: $f_1 = x$; $f_2 = x, x, -x, -x$; $f_3 = x, -x$; $f_4 = x$; $f_5 = x, x, -x, -x$; $f_6 = x$; f_7(receiver) = $x, -x, -x, x$. The sensitivity enhancement scheme of Kay is employed, i.e., the sign of G6 is inverted in concert with a 180° shift of f_6. Quadrature detection in $t_1(^{13}C)$ and $t_2(^{15}N)$ is accomplished by altering the phases f_2 and f_4, respectively, according to States-TPPI. For acquisition of central peaks derived from ^{13}C steady state magnetization, a second data set with $f_1 = -x$ is collected. The sum and the difference of the two resulting data sets generate subspectra II and I, respectively, containing the central peaks and peak pairs.



3D HACA(CO)NHN
1H
13Cα
13CO
15N
PFG
WALTZ
SEDUCE
WALTZ
SLx SLy
φ1
φ2
φ3
φ4
φ5
φ6
φ7 (rec)
y
t3
G1
G2
G3
G4
G5
G6
G7
G8

Figure 7